AMENDMENTS TO THE CLAIMS:

Please cancel Claims 1 through 65. Please add the following new Claims 66 through 107. Thus Claims 66 through 107 are pending upon entry of this preliminary amendment. Applicants reserve the right to prosecute cancelled claims in this or other applications.

Listing of Claims:

Claims 1-65 (Cancelled)

66. (New) A ligand screening method comprising the steps of:

(a) selecting a plurality of ligands not known to bind to a target protein;

(b) incubating in an assay one selected ligand and said test protein to produce a test combination;

(c) incubating said target protein in the absence of said selected ligands to produce a control combination;

(d) subjecting said test combination and said control combination to conditions to cause detectable fractions of said target protein to unfold to a measurable extent;

(e) determining the fractions of target protein in said test combination and said control combination that exists in an unfold state, a folded state, or both;

(f) comparing said determination made in step (e) between said test combination and said control combination for said selected ligand, wherein if said target protein is present in the folded state to a greater extent in said test combination than in said control combination, the ligand binds to the target protein; and

(g) repeating steps (b) to (f) with more than one thousand of selected ligands until ligands that bind to said target protein are identified.

67. (New) In the method for identifying lead compounds for possible development as pharmaceuticals by screening a plurality of test compounds that bind to a target protein, the improvement which comprises:

(a) selecting a plurality of compounds not known to bind to said target protein;

(b) admixing one selected compound with said target protein to produce a test combination;

(c) maintaining said target protein in the absence of said selected compounds to produce a control combination;

(d) subjecting said test combination and said control combination to conditions to cause detectable fractions of said target protein to unfold to a measurable extent;

(e) determining the fractions of said target protein in said test combination and said control combination that exists in an unfold state, a folded state, or both

(f) comparing said determination made in step (e) between said test combination and said control combination for said selected compound, wherein if said target protein is present in the folded state to a greater extent in said test combination than in said control combination, said compound binds to the target protein;

(g) screening in excess of one thousand selected compounds per day by performing steps (a) through (f); and

(h) selecting as a lead compound any selected compound in a test combination in which said target protein is present in the folded state to a greater extent in the test combination than in the control combination.

68. (New) A high throughput assay for identifying lead compounds for possible development as new pharmaceuticals which comprises:

(a) selecting a plurality of compounds not known to bind to said target protein;

(b) separately incubating each of said selected compounds and said target protein to produce a plurality of test combinations;

(c) incubating said target protein in the absence of said selected compounds to produce a control combination;

(d) subjecting each of said test combinations and said control combination to conditions to cause detectable fractions of said target protein to unfold to a measurable extent;

(e) determining the fractions of said target protein in each combination that exists in an unfold state, a folded state, or both;

(f) comparing the determination made in step (e) between said test combination and said control combination for said selected compounds, wherein if said target protein is present in the folded state to a greater extent in said test combination than in said control combination, said selected compound binds to the target protein;

(g) repeating steps (a) through (f) with more than 1,000 of said selected compounds; and

(h) selecting as a lead compound any selected compound from each test combination in which said target protein is present in the folded state to a greater extent in said test combination than in said control combination.

69. (New) The assay of claim 68 wherein said test ligands comprise small organic molecules.

70. (New) The assay of claim 68 which comprises using steps (a) through (h) in a large-scale, systematic high throughput screening procedure.

71. (New) The Assay of claim 68 in which between 0.1% and 1% of the total test ligands are ligands of said predetermined target protein.

72. (New) The assay of claim 68 wherein said conditions of step (d) induce the target protein to become completely denatured.

73. (New) The assay of claim 68 wherein said conditions of step (d) are sufficient to at least partially denature the target protein.

74. (New) The assay of claim 68 wherein the target protein comprises a polypeptide or protein implicated in the etiology of a disease.

75. (New). An assay for use in high throughput screening a plurality of compounds against a target protein to identify at least one of said compounds for possible development as a pharmaceutical which comprises:

(a) selecting a plurality of test compounds not known to bind to said target protein;

(b) incubating each of said test compounds and said target protein to produce a test combination;

(c) incubating said target protein in the absence of test compounds to produce a control combination;

(d) subjecting said test combination and said control combination to conditions to cause detectable fractions of said target protein to unfold to a measurable extent;

(e) determining the fractions of said target protein in each combination that exists in an unfold state, a folded state, or both;

(f) comparing the determination made in step (e) between said test combination and said control combinations for said selected ligands, wherein if said target protein is present in the folded state to a greater extent in said test combination than in said control combination, said test compound is a ligand that binds to the target protein;

(g) repeating steps (a) through (f) with said plurality of test compounds; and

(h) selecting for possible development as a pharmaceutical any test compound in a test combination in which the target protein is unfolded to a lesser extent in the test combination than in the control combination.

76. (New) A method for identifying at least one test ligand for possible development as a pharmaceutical agent from among a plurality of test ligands which comprises the steps of:

(a) providing as test ligands a plurality of ligands not known to bind to a target protein;

(b) placing at least one of said test ligands in a test well with said target protein to form a test combination;

(c) placing said target protein in a separate test well in the absence of a test ligand to form a control combination;

(d) subjecting said test combination and said control combination to conditions to cause detectable fractions of said target protein to unfold to a measurable extent;

(e) determining the fractions of said target protein in each combination that exists in an unfold state, a folded state, or both;

(f) comparing the determination made in step (e) between said test combination and said control combination for said selected ligands, wherein if said target protein is present in the folded state to a greater extent in said test combination than in said control combination, said test ligand is a ligand that binds to the target protein;

(g) repeating steps (a) through (f) for said plurality of test ligands; and

(h) selecting as a lead ligand for possible development as a pharmaceutical agent any test ligand from a test combination in which the target protein is present in the unfolded state to a greater extent in said test combination than in the control combination.

77. (New) The assay of claim 76 which comprises using said assay to screening several thousand test ligands per day.

78. (New) The assay of claim 76 which comprises subjecting said test combination and said control combination to conditions sufficient to cause a detectable fraction of the target protein to unfold in the absence of a test ligand.

79. (New) The assay of claim 76 which comprises measuring the ration of folded to unfolded target protein in the test combination and in the control combination and selecting as a lead compound any test ligand from a test combination having a higher ratio of folded to unfolded target proteins in the test combination than in said control combination.

80. (New) In the method for selecting lead compounds for development as pharmaceuticals by identifying a compound that binds to a predetermined target protein, the improvement which comprises:

(a) selecting a plurality of compounds not known to bind to said target protein;

(b) incubating each of said selected compounds and said target protein in separate containers to produce a plurality of test combinations;

(c) incubating said target protein in the absence of said selected compound in a container to produce a control combination;

(d) subjecting each of said test combinations and said control combination to conditions to cause detectable fractions of the target protein to unfold to a measurable extent;

(e) determining the fractions of said target protein in each combination that exists in an unfold state, a folded state, or both;

(f) comparing the determination made in step (e) between said test combination and said control combination for said selected compounds, wherein if said target protein is present in the folded state to a greater extent in said test combination than in said control combination, the compound binds to said target protein;

(g) repeating steps (a) through (f) rapidly with large numbers of said selected compounds; and

(h) selecting as a lead compound any selected compound in a test combination in which the target protein is present in the folded state to a greater extent than in the control combination.

81. (New) The method of claim 80 wherein the target protein is in a soluble form or bound to a solid phase matrix.

82. (New) The method of claim 66 wherein said conditions causing detectable fractions of the target protein to unfold to a measurable extent comprise heating.

83. (New) The method of claim 67 wherein said conditions causing detectable fractions of the target protein to unfold to a measurable extent comprise heating.

84. (New) The method of claim 68 wherein said conditions causing detectable fractions of the target protein to unfold to a measurable extent comprise heating.

85. (New) The method of claim 75 wherein said conditions causing detectable fractions of the target protein to unfold to a measurable extent comprise heating.

86. (New) The method of claim 76 wherein said conditions causing detectable fractions of the target protein to unfold to a measurable extent comprise heating.

87. (New) The method of claim 77 wherein said conditions causing detectable fractions of the target protein to unfold to a measurable extent comprise heating.

88. (New) The method of claim 80 wherein said conditions causing detectable fractions of the target protein to unfold to a measurable extent comprise heating.

89. (New) The method of claim 82 wherein said test ligand comprises a small organic molecule.

90. (New) The method of claim 83 wherein said test ligand comprises a small organic molecule.

91. (New) The method of claim 85 wherein said test ligand comprises a small organic molecule.

92. (New) The method of claim 86 wherein said test ligand comprises a small organic molecule.

93. (New) The method of claim 87 wherein said test ligand comprises a small organic molecule.

94. (New) The method of claim 88 wherein said test ligand comprises a small organic molecule.

95. (New) The method of claim 66 which comprises measuring the extent to which said target protein is unfoled in each of said test combination and said control combination using fluorescence spectroscopy by contacting said test combination and said control combination with a flurescence probe.

96. (New) The method of claim 67 which comprises measuring the extent to which said target protein is unfoled in each of said test combination and said control combination using fluorescence spectroscopy by contacting said test combination and said control combination with a flurescence probe.

97. (New) The method of claim 68 which comprises measuring the extent to which said target protein is unfoled in each of said test combination and said control combination using fluorescence spectroscopy by contacting said test combination and said control combination with a flurescence probe.

98. (New) The method of claim 75 which comprises measuring the extent to which said target protein is unfoled in each of said test combination and said control combination using fluorescence spectroscopy by contacting said test combination and said control combination with a flurescence probe.

99. (New) The method of claim 76 which comprises measuring the extent to which said target protein is unfoled in each of said test combination and said control combination using fluorescence spectroscopy by contacting said test combination and said control combination with a flurescence probe.

100. (New) The method of claim 77 which comprises measuring the extent to which said target protein is unfoled in each of said test combination and said control combination using fluorescence spectroscopy by contacting said test combination and said control combination with a flurescence probe.

101. (New) The method of claim 80 which comprises measuring the extent to which said target protein is unfoled in each of said test combination and said control combination using fluorescence spectroscopy by contacting said test combination and said control combination with a flurescence probe.

102. (New) The assay of claim 66 comprising identifying at least one each of said selected compound for possible development as a pharmaceutical by further determination of pharmacological characteristics of said selected compound.

103. (New) The assay of claim 67 comprising identifying at least one each of said selected compound for possible development as a pharmaceutical by further determination of pharmacological characteristics of said selected compound.

104. (New) The assay of claim 68 comprising identifying at least one each of said selected compound for possible development as a pharmaceutical by further determination of pharmacological characteristics of said selected compound.

105. (New) The assay of claim 75 comprising identifying at least one each of said selected compound for possible development as a pharmaceutical by further determination of pharmacological characteristics of said selected compound.

106. (New) The assay of claim 76 comprising identifying at least one each of said selected compound for possible development as a pharmaceutical by further determination of pharmacological characteristics of said selected compound.

107. (New) The assay of claim 80 comprising identifying at least one each of said selected compound for possible development as a pharmaceutical by further determination of pharmacological characteristics of said selected compound.

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Respectfully submitted,

Date: April 6, 2004

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